



Untersuchungsbericht

ELISA-Test Allergenbestimmung

GETIFIX Franchise GmbH

Haferwende 1

D-28357 Bremen

Untersuchungsergebnis

Probenahme : Getifix Myzel- und Sporenvernichter MuS

Proben Nr. : 204-767

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Internet: <http://www.umweltanalytik.com>

In Zusammenarbeit mit:

Institut für Umweltkrankheiten

Im Kurpark 1

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Tel.: 05624/8061

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Seite: 1

Allergene	Probenlösung ng/ml	Ausgangskonzentration ng/ml
Asp f1	5	250

< = nicht nachweisbar, unter der Nachweisgrenze

Untersuchungsmethode :

Zu 450 µl der Probenlösung (Getifix Myzel- und Sporenvernichter MuS) werden 50 µl des Allergenstandards Asp f1 (Indoor Biotechnologies: ST-AF1 / ST-AA1) addiert.

Die Bestimmung des Substanzgehaltes erfolgt mittels eines allergenspezifischen Immunoassay-Verfahrens (ELISA-Test) i.A. durch Indoor Biotechnologies, Inc, USA (Methodenbeschreibung: siehe Anlage).

Bewertung :

In der Probenlösung (Getifix Myzel- und Sporenvernichter MuS) mit Standardaddition des Schimmelpilzallergens Asp f1 konnte ein um 98% reduzierter Allergengehalt gegenüber der Ausgangskonzentration festgestellt werden.

Kassel, den 20.04.2005

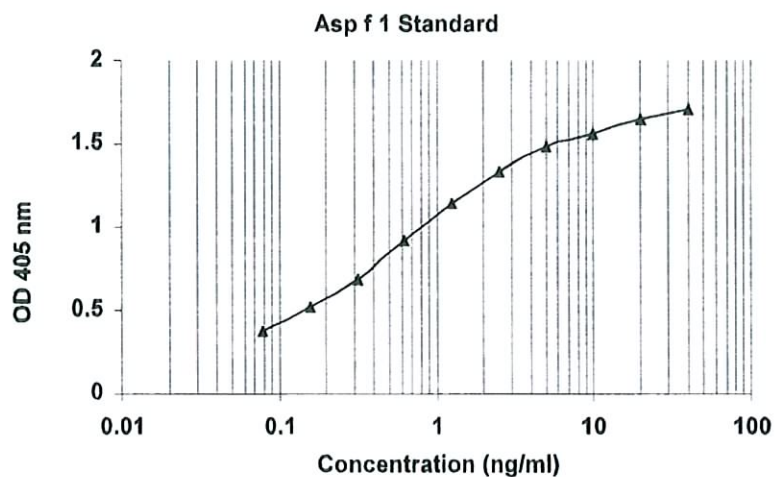

Dipl.-Ing. Holger Oetzel

Dieser Bericht umfaßt 1 Seite(n) !



Asp f 1 ELISA kit

Product Code: EL-AF1



Content:

- ◆ Vial 1 (red top) 100 μ l
Monoclonal antibody 4A6
Concentration: 10mg/ml in PBS
- ◆ Vial 2 (white top) 400 μ l
Asp f 1 Standard
Concentration: 400ng/ml
- ◆ Vial 3 (brown) 100 μ l
Polyclonal rabbit anti-Asp f 1 antibody
Dilute: 1:1000 for use

Storage: All reagents should be stored at 4°C

Developed and manufactured by:

Indoor Biotechnologies, Inc.
1216 Harris Street
Charlottesville, VA 22903
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For Research Use Only: Not for Diagnostic or Therapeutic Use

**ELISA protocol for Asp f 1: Product Code EL-AF1**

1. Anti-Asp f 1 mAb 4A6 is supplied HPLC purified as a stock solution in PBS (Product Code: MA-4A6). Dilute the mAb 4A6 1/1000 (i.e. 10µl/10ml) in 50mM carbonate-bicarbonate buffer, pH 9.6. Coat polystyrene microtiter wells (NUNC Maxisorp Cert. NUNC catalog # 439454, Fisher Catalog #12565135) with 100µl of the diluted mAb 4A6 per well. Incubate overnight at 4°C.
 2. Wash wells 3x with PBS-0.05% Tween 20, pH 7.4 (PBS-T). Incubate for 30 min. at room temperature with 100µl 1% BSA PBS-T. Wash 3x with PBS-T.
 3. Add 100µl of diluted allergen standard, house dust or air filter samples. Incubate for 1 hour at room temperature.
 - 3a) Make a Asp f 1 control curve using doubling dilutions of the allergen standard (Product Code: ST-AF1): The control curve dilutions are from 40 - 0.04ng/ml Asp f 1. Pipette 20µl Asp f 1 standard into 180µl 1% BSA PBS-T into wells A1 and B1 of the ELISA plate. Mix well and transfer 100µl across the plate into 100µl 1% BSA PBS-T diluent to make 10 serial doubling dilutions. Wells A11, B11, A12 and B12 should contain only 1% BSA PBS-T as blanks.
 - 3b) House dust samples are routinely diluted two-fold from 1/10-1/80.
 4. Wash wells 3x with PBS-T and add 100µl diluted Rabbit anti-Asp f 1 antibody (Product Code: PA-AF1). The antibody solution contains 50% glycerol and should be diluted 1/1000 (i.e. 10µl/10ml) in 1%BSA-PBS-T. Incubate for 1 hour at room temperature.
 5. Wash wells 3x with PBS-T and add 100µl diluted Peroxidase conjugated Goat anti Rabbit IgG (Jackson Laboratories Cat.# 111-036-046, reconstituted in 1ml distilled water and 1ml glycerol). The reconstituted Goat anti Rabbit IgG should be diluted 1/1000 (i.e. 10µl/10ml).
 6. Wash wells 3x with PBS-T and develop the assays by adding 100µl 1mM ABTS in 70mM citrate phosphate buffer, pH 4.2 containing a 1/1000 dilution of 30% H₂O₂ (i.e. 10µl/10ml ABTS). Read the plate when the optical density at 405nm reaches 2.0-2.4.
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References:

1. Arruda, LK, Platts-Mills, TAE, Fox, JW and Chapman, MD (1990). *Aspergillus fumigatus* allergen I, a major IgE binding protein, is a member of the mitogillin family of cytotoxins. *J. Exp. Med.*, 172:1529-1532.
2. Arruda LK, Platts-Mills TAE, Longbottom JL, Chapman MD (1992). *Aspergillus fumigatus*: Identification of 16kd, 18kd and 45kd antigens recognized by human IgG and IgE antibodies and murine monoclonal antibodies. *J. Allergy Clin. Immunol.* 89:1166-1176.
3. Arruda LK, Mann BJ, Chapman MD (1992). Selective expression of a major allergen and cytotoxin, Asp f I, in *Aspergillus fumigatus*. *J. Immunol.*149:3354-3359.
4. Sporik RB, Arruda LK, Woodfolk J, Chapman MD, Platts-Mills TAE. (1993). Environmental exposure to *Aspergillus fumigatus* allergen 1 (Asp f I). *Clin Exp Allergy* 23:326-331.
5. El-Dahr JM, Fink R, Selden R, Arruda LK, Platts-Mills TAE, Heymann PW. (1994). Development of immune responses to *Aspergillus* at an early age in children with cystic fibrosis. *Am J Respir Crit Care Med* 150:1513-1518.



SAMPLE
Certificate of Analysis

Antibody: Monoclonal anti Asp f 1 mAb, 4A6
Immunogen: Asp f 1
Isotype: Mouse IgG1
Specificity: Binds to a common epitope on *Aspergillus fumigatus* allergen, Asp f 1.
Purification: 50% saturated ammonium sulfate fraction of ascites, dialyzed against BBS pH 8.0 for 48 hours.
Concentration: 10mg/ml in phosphate buffered saline, pH 7.2. Based on A280 for IgG (1.42= 1mg/ml) 0.22µm filtered, preservative free.
Lot Number: 2420

Antibody: The rabbit anti-*A.fumigatus* antibody is a polyclonal antiserum raised against partially purified Asp f 1 (a fraction eluted with 2M NaCl from a phenyl-sepharose column) (1, 2).
Specificity: The antiserum contains IgG antibodies to Asp f 1 as well as IgG antibodies to other *Aspergillus* allergens.
Activity: The antiserum has been diluted in phosphate buffered saline, pH 7.4, containing 1% BSA-50%glycerol. The antiserum has been filtered and should be diluted 1/1000 for use in Asp f 1 assays.
Lot Number: 2452

Allergen Standard: Asp f 1
Composition: *Aspergillus* extract prepared in 1% BSA/50% glycerol/PBS. pH 7.4
Concentration: 400ng/ml Asp f 1.
Calibration: Asp f 1 was purified from *Aspergillus* extract by affinity chromatography using anti-Asp f 1 monoclonal antibody 4A6. The protein concentration was determined by ELISA.
Lot Number: 2521